Effect of Cations Upon Recovery of the Guinea Pig Gut From Inhibition by Antihistaminics

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When the guinea pig ileum suspended in Tyrode is exposed to the action of antihistamine substances (Pyribenzamine, Neo-antergan, Antistin, Benadryl, etc.) for exactly 1 min and tested with a standard dose of histamine, every 1.5 min, after washing out the inhibitor, the course of recovery can be followed quantitatively. In a separate paper (*4*) we have defined the index R_{50} as the time in seconds necessary for a 50% recovery to be attained. Assuming that the response of the muscle to a certain dose of histamine is a measure of the number of receptors available (*2, 3*), one can calculate the constant *k*, of a first-order reaction in the assumption that breaking up of the inhibitor-receptor complex follows the law of a monomolecular reaction. When this is done, we can verify that *k* increases continuously as recovery proceeds and that it follows the same law of increment as the percentage of response (P) during recovery. If *k* is plotted against P, a straight line is obtained, indicating that the newly formed receptors "catalyze" or otherwise influence formation or regeneration of the receptors. A plausible explanation for this fact would be that inhibition produced by the mentioned agents depends upon destruction of a metabolite, the presence of which is necessary for the normal interaction between active drug and the specific receptors. Regeneration of receptors would depend upon the autocatalytic reconstruction of the affected metabolite. An alternative explanation was suggested by the study of ions upon this phenomenon of recovery, as we see in continuation.

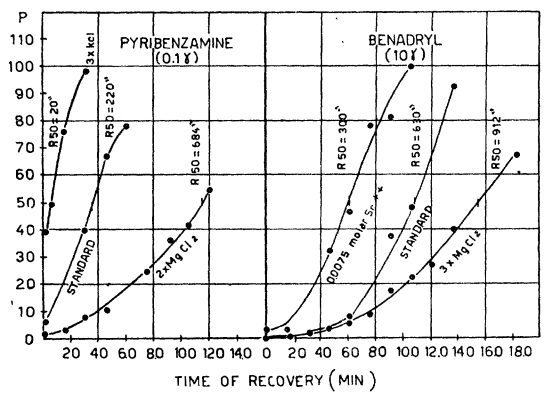

FIG. 1. Effect of increasing the concentrations of $MgCl_2$ and KCl of the standard Tyrode solution, as well as of adding 0.0075 molar of $SrCl_2$, upon recovery from inhibition of a piece of guinea pig ileum by Pyribenzamine and Benadryl. Ordinate: percentages of response to 0.2 cc of histamine, 1 : 2 millions.

Since recovery from inhibition produced by high doses of acetylcholine and histamine follows the same law as for other inhibitors, the work of Cantoni and Eastman (*1*) on the effect of KCl in suppressing refractoriness after high doses of histamine and acetylcholine acquired a timely interest. We decided, therefore, to study the effect of cations upon recovery of the gut from inhibition by Neo-antergan, Pyribenzamine, Benadryl, Antistin, Trasentin, etc. In all cases, 2–5 times the concentration of K^+ of the standard Tyrode solution produced a considerable accelerating effect. Mg^{++}, on the contrary, had a strong slowing-down effect when added in concentrations up to 2 or 3 times that of the Tyrode solution. Ca^{++} had a lesser effect than Mg^{++}, and, in some cases, it even had a slight accelerating effect, but in general its depressing effect was predominant. Surprisingly enough, Sr^{++}, even when present in the low concentration of 0.0025 molar, had a definite accelerating effect. Fig. 1 shows typical experiments with Pyribenzamine and Benadryl.

The accelerating effect of potassium and, in minor scale, of strontium must be understood as a somewhat specific effect and does not appear to depend upon equilibrium with calcium or magnesium. If 3 times Mg^{++} is combined with 3 times K^+, the accelerating effect of K^+ predominates; on the other hand, when the concentration of Ca^{++} is lowered to ½ of the standard, there occurs a slowing down of recovery, of the same extent as can be seen when K^+ is lowered to half of the standard. Sometimes, when calcium or potassium is lowered to half of the standard, complete recovery becomes impossible, but full activity can be rapidly restored by switching to a high potassium Tyrode. Absence of Mg^{++} in the perfusing Tyrode had slight or no effect upon the rate of recovery.

The effects of K^+, Ca^{++}, and Mg^{++} were similar but quantitatively different when assayed upon recovery from inhibition produced by high doses of acetylcholine and histamine. K^+ had the same strong effect, and therefore we could confirm Cantoni and Eastman (*1*). Ca^{++} had a stronger dilatory effect than that referred to above, although Mg^{++} was considerably less effective upon this kind of inhibition than toward inhibition produced by antihistaminics. Sr^{++}, which was so clearly accelerating upon recovery from inhibition by antihistaminics, had a moderate but definite depressant effect upon recovery from high doses of acetylcholine and histamine. This discrepancy is not well understood up to now and is being investigated.

From these studies it appeared likely that integrity of specific receptors depends upon a sort of polarization of the ionic layers supposedly present at the muscle surface and that inhibition or inactivation of receptors might depend upon disturbance of these ionic arrangements, the importance of which for muscle activity is well known. It has been shown, for instance, that myosin strongly adsorbs positive ions, the bivalent cations being adsorbed first. A third layer of ATP would establish conditions for muscle activity (*5*). Phenomena of autocatalytic interchange of these ionic layers has also been mentioned by Szent-Györgyi, although no single scheme might fit exactly with the phenomena here described.

The practical implications of these findings are obvious, since high concentrations of K^+ might contribute to decrease the effects of antihistaminics; on the other hand, a combination of Mg^{++} and Ca^{++} might contribute to strengthen the effect of these important therapeutic agents.

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Stubble Mulch Studies: Effect of Sweetclover Extract on Corn Germination

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Numerous studies have shown the varied influence of plant and microbial extracts and decomposition products on the growth of plants and microorganisms (*1–5*). In the rapidly expanding system of stubble mulch farming, plant materials are being used for mulching the soil to protect it against excessive runoff and erosion by wind and water. Under certain conditions, some mulching materials have been observed to reduce the stand and growth of corn. In the field, sweetclover has not been observed to influence stands of corn adversely. In fact, corn following sweetclover has been much more productive than that on land without legumes. This improved or stimulated growth usually has been attributed to the increased amount of available nitrogen released upon the decomposition of sweetclover residues.

The effect on germination of water extracts of several different plant materials including sweetclover was tested. Sweetclover hay that had been cut when the plants were about 18″–24″ high was used in the tests herein reported. Portions of the hay were cut into short lengths and placed in flasks with varying proportions of water. The corn seeds were then placed in the flasks and soaked in the sweetclover-water mixture for 24 hrs. At the end of the soaking period 5 seeds from each concentration of sweetclover extract were placed on an agar medium[1] in Petri dishes in quadruplicate. The plates were incubated at room temperature. After 3 days the root and top growth of the corn seedlings were measured and compared with those of seeds soaked in distilled water. The sweetclover had a more depressing effect on germination and seedling growth than did alfalfa, wheat straw, or oat straw. At times wheat straw in the laboratory has given no effect, and in other tests a stimulation of growth. The results with sweetclover are shown in Table 1. Even at a dilution of 1 part of sweetclover to 100 parts of water the growth of the corn seedlings was reduced.

[1] The medium (Crone's) contained all essential mineral nutrients except nitrogen, and 1.5% agar.

TABLE 1

INFLUENCE ON GERMINATION AND GROWTH OF SOAKING CORN SEEDS FOR 24 HRS WITH SWEETCLOVER EXTRACT

	Parts of plant material to distilled water	Germination (%)	Seedling growth after 3 days (length in cm)*	
			Tops	Roots
Sweetclover	1– 5	33	0.3	0.8
"	1– 10	52	0.5	0.9
"	1– 20	75	0.7	1.7
"	1– 80	92	1.8	3.8
"	1–100	87	1.6	4.0
Control		95	2.8	6.4

* Based on the number of germinated seeds.

The results with corn seeds soaked in different concentrations of coumarin in distilled water are shown in Table 2. It appears from these results that the coumarin in sweetclover may be one of the constituents of sweetclover that inhibited germination and growth of the corn seedlings.

TABLE 2

THE INFLUENCE ON GERMINATION AND GROWTH OF SOAKING CORN SEEDS IN COUMARIN SOLUTION FOR 24 HRS

Grams of coumarin in 100 ml of water	Germination (%)	Seedling growth after 3 days (length in cm)	
		Tops	Roots
0.125	0	0	0
0.062	0	0	0
0.031	12	0.3	0.4
0.015	42	0.4	0.5
0.007	90	1.2	4.2
None	95	2.9	7.6

From these and other results obtained in similar tests it appears that many plant residues contain substances which, under certain conditions, retard or inhibit germination and growth of plants in the seedling stage. This fact may have far-reaching implications with respect to the whole question of plant growth. However, the effect of these substances when in the soil and exposed to innumerable microbiological reactions may be entirely different than when in relatively pure cultures.

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